

Maternal adiposity:

Associations with gestational diabetes and
neonatal fat in a multi-ethnic population

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Oslo, Christine Sommer

ABBREVIATIONS

ADA	American Diabetes Association
BIA	Bioelectrical impedance analysis
BMI	Body Mass Index
CI	Confidence interval
CT	Computed Tomography
DOHaD	Developmental Origins of Health and Disease
DXA	Dual X-ray Absorptiometry
GDM	Gestational Diabetes Mellitus
HAPO	Hyperglycemia and Adverse Outcomes
HDL	High Density Lipoprotein
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
IADPSG	International Association of the Diabetes and Pregnancy Study Groups
IOM	Institute of Medicine
LDL	Low Density Lipoprotein
MRI	Magnetic Resonance Imaging
NEFA	Non-Esterified Fatty Acids
OGTT	Oral Glucose Tolerance Test
OR	Odds ratio
s-leptin	serum leptin
SD	Standard Deviation
T2DM	Type 2 Diabetes Mellitus
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization

LIST OF PAPERS

- I: Sommer C, Mørkrid K, Jenum AK, Sletner L, Mosdøl A, Birkeland KI. Weight gain, total fat gain and regional fat gain during pregnancy and the association with gestational diabetes: a population-based cohort study. *International Journal of Obesity* (London), 2014 Jan;38(1):76-81. doi: 10.1038/ijo.2013.185.
- II: Sommer C, Jenum AK, Waage CW, Mørkrid K, Sletner L, Birkeland K. Ethnic Differences in BMI, Subcutaneous Fat and Serum Leptin Levels During and After Pregnancy and Risk of Gestational Diabetes. *European Journal of Endocrinology*, 2015, Jun;172(6):649-656. doi:10.1530/EJE-15-0060.
- III: Sommer C, Sletner L, Mørkrid K, Jenum AK, Birkeland KI. Effects of early pregnancy BMI, mid-gestational weight gain, glucose and lipid levels in pregnancy on offspring's birth weight and subcutaneous fat. A population-based cohort study. *BMC Pregnancy and Childbirth*, 2015, Apr;3;15(1):84.. [Epub ahead of print]. doi:10.1186/s12884-015-0512-5

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INTRODUCTION

The proportion of overweight and obese individuals has increased during the last decades ^{1,2}. In the European region, the prevalence of overweight and obese women aged 15 years or above was 52 % in 2010 (according to the WHO global infobase). Obesity is associated with metabolic disturbances, higher morbidity ³ and mortality ⁴. Obesity is a major risk factor for type 2 diabetes (T2DM) ⁵, and simultaneously with the increasing prevalence of obesity, there has been a rise in the incidence of T2DM ⁶.

Immigrants to western societies seem to be disproportionately affected with T2DM ⁷⁻⁹. The disease occurs at an earlier age in Asian immigrants ¹⁰, and a study from Norway found an earlier onset also in Middle Eastern immigrants compared to the host population ¹¹. Onset of T2DM at a younger age threatens both the health of the individual ¹² as well as the economic burden ¹³, since it may result in more late complications.

Maternal pre-existing diabetes increases risk of future obesity and diabetes in the offspring ¹⁴⁻¹⁶. Although maternal pre-existing diabetes is undoubtedly more detrimental to the offspring's health than less severe hyperglycaemia, such as gestational diabetes (GDM), the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study found a continuous relationship between maternal glucose levels and several pregnancy outcomes ¹⁷. In utero exposure to any degree of hyperglycaemia may therefore influence the metabolic health and the growth of the foetus, and be an important risk factor for later T2DM for both the mother ¹⁸ and her offspring ¹⁶.

The present thesis explores ethnic differences in adiposity and weight gain during pregnancy and risk of maternal hyperglycaemia, which might affect the offspring's birth weight and subcutaneous fat, and thereby possibly the future risk of obesity and diabetes (Figure 1). This thesis focuses on adiposity and diabetes, although other chronic diseases, such as cardiovascular disease, could have been just as relevant.

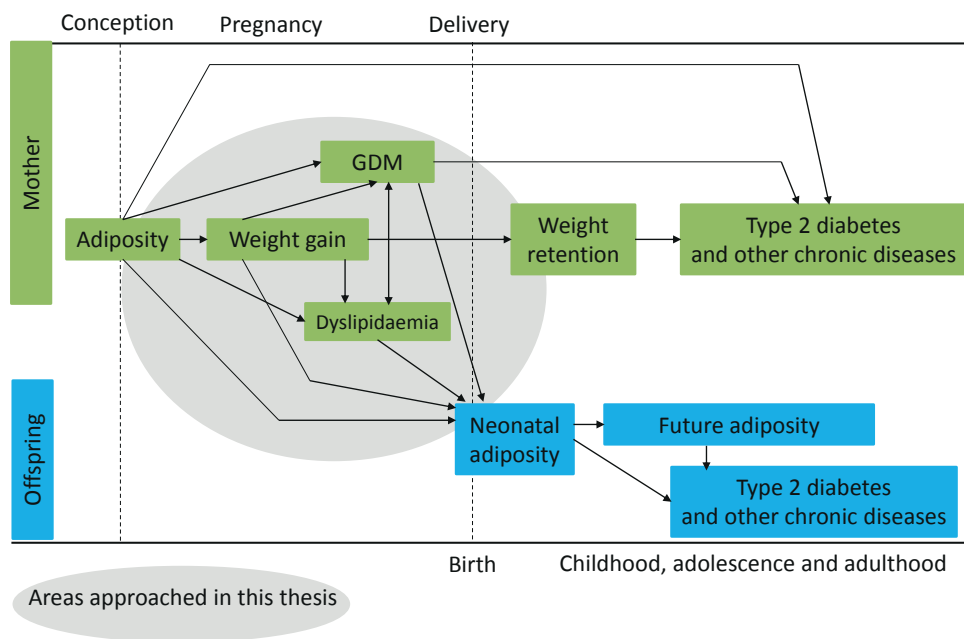


Figure 1. Conceptual framework of the thesis.

If pre-pregnant adiposity, gestational weight gain, proportion of women with GDM, maternal dyslipidaemia and postpartum weight retention differs across ethnicity, this may explain some of the ethnic differences in future risk of type 2 diabetes both in the mother and in her offspring.

1.1 MATERNAL ADIPOSITY

Adiposity refers to excessive amounts of adipose tissue. Adipose tissue is mainly storage of excess energy, although it also provides some protection and insulation of the body. Recently, adipose tissue has additionally been recognized as a potent endocrine and paracrine organ¹⁹, with its production of hormones such as leptin, adiponectin¹⁹ and cytokines such as tumor necrosis factor alpha and interleukin 6²⁰. Adiposity, no matter how it is measured, is strongly associated with morbidity²¹, insulin resistance⁵ and T2DM²².

Maternal adiposity is strongly associated with increased risk of GDM²³. Maternal adiposity is also associated with several pregnancy related health outcomes for the mother and her offspring, such as increased risk of large for gestational age babies²⁴, foetal death, stillbirth and infant death²⁵ and future obesity in the offspring²⁶.

1.1.1 Fat localization

Adipose tissue biology depends on its localization. For example, the degree of insulin resistance in lean individuals vary with degree of central adiposity⁵. Subcutaneous fat is generally considered a healthy way of storing fat due to its function as a “metabolic sink” that buffers energy excess²⁷, but subcutaneous fat may lose its ability to store fat in extreme energy excess²⁸.

Central body fat refers to fat situated in the abdomen, both visceral fat (intra-abdominal) and upper body subcutaneous fat (Figure 2).

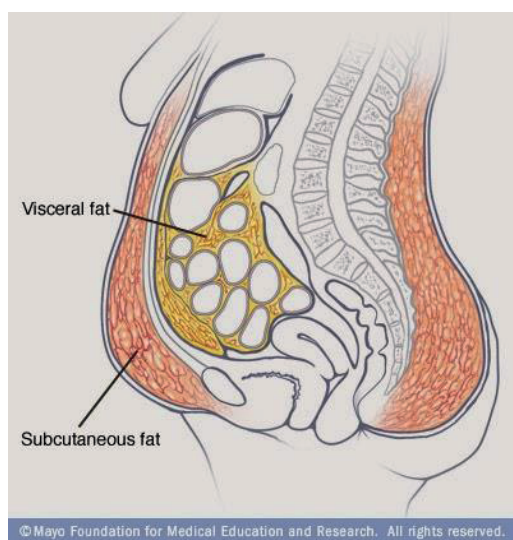


Figure 2. Illustration of central fat, consisting of both visceral and subcutaneous fat.
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Visceral fat can be further divided into omental and mesenteric fat, while subcutaneous fat is divided into deep and superficial subcutaneous fat²⁹. Accumulation of deep subcutaneous fat is correlated with accumulation of visceral fat³⁰. Lower body fat is often divided into gluteal fat, subcutaneous leg fat and intramuscular fat²⁹. Central fat is thought to be more metabolically active than fat situated elsewhere and is associated with increased risk of T2DM^{31,32}. The role of central fat in pregnancy, however, has been scarcely studied.

1.1.2 Measurement of adiposity

To estimate the amount and distribution of adipose tissue in living humans, several methods have been described (Table 1). Underwater weighing, also known as densitometry, was long considered the gold standard until technological advances provided computed tomography (CT) and magnetic resonance imaging (MRI), which both accurately quantify percent body fat, visceral and subcutaneous fat. However, the most accurate methods are expensive, not portable and time consuming, making them unsuited for large epidemiological studies. Epidemiological studies, with relatively large number of participants, generally demands methods that are quick and easy to perform, preferably inexpensive and without large measurement error. Hence, in epidemiological studies there is frequent use of surrogate measures to estimate the degree of adiposity (Table 1).

However, not all methods are feasible in pregnancy. Dual x-ray absorptiometry (DXA) and CT are not recommended in pregnancy due to ionizing radiation. MRI may be difficult in obese, or even in overweight women late in pregnancy, due to the small diameter (usually 60 cm) of the machine. Waist circumference, as a measure of central adiposity, loses its relevance in pregnant women with the growing belly. In addition, several methods are challenged by physiological changes natural to pregnancy, such as increased body water due to amniotic fluid and increased amounts of extracellular fluid. Underwater weighing usually overestimates fat mass in pregnancy, as the extra weight added in pregnancy will have a lower density than the lean tissue in non-pregnant³³. The dilution method, on the other hand, underestimate fat mass, as the water tracers “escape” into gestational tissues, and leaves a falsely low concentration indicating a high proportion of fat-free mass³³. Some correcting equations have been made for both methods, but it is generally advised to combine the two if high accuracy is demanded. In epidemiological studies of pregnant women, the method choices are therefore limited.

Table 1. Common methods for assessment of body composition.

Method	Description	Strengths and limitations
Reference methods		
Dissection	Dissection of an individual, collecting adipose tissue and weighing was long considered the absolute gold standard	Obviously accurate, but not available in living individuals.
Underwater weighing (Densitometry)	The individual is weighed both in air and underwater. Percent body fat is calculated with equations based on the principle that water has higher density than fat	Was for a long time considered the gold standard. Time-consuming, cannot assess body fat distribution, less value in pregnancy.
Air-displacement plethysmography	Uses the same principle as underwater weighing, but measures air displacement in an enclosed chamber. Highly reliable and valid as compared to underwater weighing ³⁴ .	Much quicker and more comfortable than underwater weighing. Cannot assess body fat distribution, scarcely available-
Dilution method (hydrometry)	Measure total body water using isotopes. Based on the assumption that the ratio between body water and fat-free mass is stable. Validated equations are used to calculate total body water and fat-free mass	Relatively inexpensive, simple and safe. The ratio between body water and fat-free mass may, however, not be stable, especially in some situations such as certain diseases, weight loss, pregnancy etc.
Dual x-ray absorptiometry (DXA)	Based on two x-ray beams, which are absorbed differently by bone mineral tissue and soft tissue. Fat-free mass, fat mass and bone mineral density is estimated for arms, legs and trunk	High reproducibility and accuracy. Expensive and not portable. Not suited in pregnancy due to ionizing radiation (although low).
Computed tomography (CT)/magnetic resonance imaging (MRI)	High-resolution images of organs and tissues. Highly accurate assessment of body composition and regional fat	Considered gold standard. Can differentiate between different regions of fat. Difficult in morbidly obese due to the diameter of the machines (usually 70 cm in CT and 60 cm in MRI), can cause a sense of claustrophobia (especially MRI). Expensive, not portable. CT is not suited in pregnancy due to ionizing radiation.
Surrogate measures		
Body mass index (BMI)	Weight divided by squared height. Surrogate for body fat.	Correlates well with total body fat from reference methods at population level, poor performance at individual level. Simple and highly manageable in epidemiologic studies. Performs

Waist circumference, waist-to-hip ratio, waist-to-height ratio	Surrogate for central obesity.	differently across ethnic groups. Correlates well with central fat from reference methods. Simple and manageable in epidemiologic studies. Problematic in pregnancy, as the waist naturally grows.
Skinfold thickness	Skinfold thickness is measured with a caliper in certain sites; triceps, biceps, subscapular, abdomen, suprailiac, thigh. Surrogate for body fat and regional distribution. Total body fat can be calculated based on equations	Correlate well with body fat. Simple and manageable in epidemiologic studies. Inter-rater variability is usually high, resulting in low precision.
Bioelectrical impedance analysis (BIA)	Measures resistance to an electrical current that is sent through the body to estimate total body water. Total body water, fat mass and fat-free mass is estimated based on in-built equations.	Correlates well with reference methods. Relatively inexpensive, simple and manageable in epidemiologic studies. Is affected by body structure, hydration and disease status. Some claim that the performance may be lower in some ethnic groups, as the in-built equations are based on large sample consisting mainly of Western populations.
Biomarkers		
Leptin	Reflects body fat. Some evidence suggest that free (unbound) serum leptin is excreted in exact proportions to fat mass. Subcutaneous fat is thought to correlate better with leptin than visceral fat. Large adipocytes are thought to excrete more leptin than normal sized adipocytes.	Cannot accurately estimate fat mass, but is considered a good indicator. Simple and manageable in epidemiologic studies. Cannot assess body fat distribution

Table adapted from F.B. Hu ³⁵.

Body mass index

Body mass index (BMI) is the most widespread surrogate measure of adiposity, and is easily calculated based on weight and height (Weight divided by height squared). BMI is widely used in epidemiological studies and is considered to provide accurate estimates of body composition in adults ³⁶. The World Health Organization (WHO) has developed BMI categories based on risk of morbidity (Table 2) ³⁷.

In pregnancy, BMI can be used as an indicator of adiposity in a continuous matter, but the BMI categories will be more and more misleading as the pregnancy progresses, due to the natural weight gain.

Table 2. Classification of BMI according to the WHO ³⁷.

Classification	BMI cut-offs
Underweight	<18.50
Normal weight	18.50-24.99
Overweight	25.00- 29.99
Obese	≥30.00
Obese, class I	30.00-34.99
Obese, class II	35.00-39.99
Obese, class III	≥40.00

Skinfolds

Measurements of skinfold thickness are often used as an indicator of adiposity and body fat distribution ³⁵. Measurements of skinfold thickness are relatively easy to perform in large cohorts. Skinfold thickness is measured at predefined sites, usually triceps, biceps, subscapular, suprailiac, abdomen and thigh. Although the within observer (intra-rater) reliability is usually acceptable, the between observer (inter-rater) variation is usually larger ³⁸, which may result in imprecision, low reliability and reproducibility. The measurement error is generally greater for skinfold thickness measurements than for other anthropometric variables ³⁵. Several equations exist to estimate total body fat based on skinfold thickness ³⁵, although their estimation performance and validity vary across different populations, e.g. ethnic groups. In pregnancy, the measurement of skinfold thickness in the abdominal site becomes more difficult as the pregnancy progresses.

Bioelectrical impedance analysis

The bioelectrical impedance analysis (BIA) sends electrical currents through the body between electrodes, and thereafter measures the resistance (ohm) given by the body before the electrical current returns to the other electrode. Based on in-built equations, using age, body weight, height and resistance in the body, the BIA scale estimates total body water, muscle mass and fat mass. The BIA scales usually measure the resistance via four electrodes at the feet that generally measures the resistance in the underpart of the body, or via eight electrodes, two for each foot and two for each hand, that measure resistance in the entire body

(except from the head). As BIA scales with eight electrodes measure the resistance both in the lower and upper part of the body, they are able to estimate central fat mass in addition to total fat mass. The estimation of total body water during pregnancy by BIA scales has been found valid during pregnancy³⁹. However, the use of BIA scales for estimation of body fat in pregnancy has, as far as I am aware of, not been formally validated. As the variation in body water to fat-free mass ratio between individuals increases throughout pregnancy⁴⁰, it is possible that the estimation of body fat is less accurate in late pregnancy. However, the mean body water to fat-free mass ratio is the same throughout pregnancy⁴⁰ indicating that on a population level, the BIA should perform well. The performance of fat mass in pregnancy using BIA has been compared with underwater weighing (known to overestimate fat mass in pregnancy) and the dilution method (known to underestimate fat mass in pregnancy). BIA correlated with both methods, suggesting that the risk of systematic bias with BIA is low³³.

Biomarkers

Adipose tissue secretes several adipokines, which could potentially function as biomarkers of fat mass. Both leptin and adiponectin has been associated with GDM, while the evidence for association between GDM and other adipokines is sparse⁴¹. Adiponectin is inversely related to fat mass, has been associated with insulin-sensitizing effects and is merely considered a marker of the metabolic syndrome⁴².

Leptin is a hormone that is secreted by adipose tissue and its discovery was the first evidence of major endocrine properties of adipose tissue¹⁹. Leptin is known as the satiety hormone, as high concentrations activate leptin receptors in the hypothalamus which inhibits hunger. Leptin is also involved in adjustment of energy expenditure. Paradoxically, obese individuals have high levels of leptin⁴³, indicating that the high leptin levels fail to inhibit hunger and to increase energy expenditure, possibly due to a reduction in leptin receptors or a general lack of leptin's effect. Serum leptin (s-leptin) is exponentially related to fat mass^{44,45}, and seems to correlate better with subcutaneous fat than with visceral fat in women^{46,47}. Consistently, levels of leptin mRNA are higher in subcutaneous than in visceral adipocytes^{48,49}, especially in women⁴⁹. Recently, s-leptin is increasingly being used as an indicator of adiposity. In pregnancy, leptin is additionally produced and secreted by the placenta, leading to higher levels with progressing pregnancy, followed by a rapid decline to pre-pregnancy levels after delivery^{50,51}. Hence, s-leptin levels will be a better indicator of adiposity in early pregnancy,

when the placenta is not yet fully developed, and after delivery when the placenta is no longer present.

1.1.3 Measurement of adiposity and ethnicity

The use of BMI across ethnicity may represent a challenge, as the relative amount of lean and fat tissue may differ across populations. Asians have been found to have a higher amount of adipose tissue relative to their BMI than “whites”⁵². South Asians have higher levels of leptin for the same BMI compared to Europeans^{53,54}. In accordance with these findings, Asians have a considerably higher risk of diabetes at the same BMI level^{55,56}. Therefore, lower cut-off levels to define overweight and obesity in certain ethnic groups have been proposed and later accepted by the WHO^{57,58}. However, as proper cut-off levels varies within Asian populations, the WHO do not operate with different recommendations, but encourage the use of additional cut-offs (Table 3) for reporting purposes^{37,58}.

Table 3. Additional cut-offs for BMI according to the WHO³⁷.

Classification	Principal BMI cut-offs	Additional cut-offs¹
Underweight	<18.50	
Normal weight	18.50-24.99	18.50-22.99 23.00-24.99
Overweight	25.00- 29.99	25.00- 27.49 27.50-29.99
Obese	≥30.00	
Obese, class I	30.00-34.99	30.00-32.49 32.50-34.99
Obese, class II	35.00-39.99	35.00-37.49 37.50-39.99
Obese, class III	≥40.00	

¹ Additional cut-offs for reporting purposes

The validity of BIA scales across ethnic groups is compromised by ethnic differences in how well the equations perform. Several studies have derived separate equations for certain ethnic groups⁵⁹⁻⁶³, validated against methods that are considered more robust across ethnicity, such as DXA. However, the ethnicity specific equations are almost exclusively based on studies with small samples, and ethnicity specific equations are not available for all ethnic groups. BIA scales have been found to underestimate fat mass in female Asians^{59,62,64} and to underestimate⁵⁹ and overestimate⁶² fat mass in black females.

Although most methods for body fat assessment across ethnicity are somewhat challenged by their dependence on equations based on a certain population, methods such as MRI and CT are considered valid across ethnic groups. Our collaborators in Pune, India, found that subscapular and suprailiac skinfolds, as indicators of central deposition of fat, predicted glycaemia and insulin resistance while central fat measurements from MRI, DXA or CT did not substantially improve the prediction⁶⁵, suggesting that skinfolds may be an easy way to assess detrimental adiposity in South Asians.

1.1.4 Maternal adiposity and ethnicity

The “Adipose tissue overflow” hypothesis—suggesting that South Asians have lower capacity to store superficial subcutaneous fat leading to deposition of visceral fat when facing energy excess—has been proposed as an explanation for the higher diabetes risk observed in South Asians⁶⁶. Studies exploring ethnic differences in adiposity are scarce, and the studies mostly rely on BMI as a surrogate measure. Among ethnic minorities living in Europe, women from the Middle East and Africa seem to have a higher degree of overweight and obesity prior to pregnancy, while Asian women tend to have a lower BMI compared to the host population⁶⁷.

1.2 GESTATIONAL WEIGHT GAIN

Not only pre-existing overweight and obesity, but also excessive gestational weight gain during pregnancy may be detrimental to health. The gestational weight gain consists of several components; the weight of the foetus, uterus, placenta, mammary glands, intra- and extracellular fluid and adipose tissue⁶⁸. The individual variation in gestational weight gain is mainly thought to be due to differences in fat accretion and total body water. The variation between individuals in total body water is higher in the later stages of pregnancy than in early pregnancy.

For the mother, excessive gestational weight gain increases the risk of a number of pregnancy-related complications, risk of GDM^{68,69} and later obesity and T2DM^{68,70}. For the offspring, excessive gestational weight gain increases the risk of being born large for gestational age⁷¹ and obesity in the childhood, adolescence and in adult life^{68,72}. Moreover, some studies suggest that excessive gestational weight gain may increase the cardiovascular risk of offspring in adult life, but these findings are inconsistent⁷². A too low weight gain in

the mother due to nutritional restrictions, on the other hand, seems to increase the risk of the offspring to be born small for gestational age ⁷¹, and, paradoxically, for obesity, cardiovascular disease and T2DM in adulthood ^{68,72}.

The U.S. Institute of Medicine (IOM) has issued guidelines for what they consider to be adequate weight gain during pregnancy (Table 4) ⁶⁸. These recommendations suggest different intervals of weight gain dependent on pre-pregnant BMI, with the aim to reduce the risk of adverse consequences for both mother and offspring ⁷⁰⁻⁷². The IOM guidelines have been criticized for recommending a too high weight gain, especially for women in obesity classes II and III ⁷³. However, the committee members claim that more studies are needed to provide obesity class specific guidelines ⁷⁴. It is important to not recommend a too low maternal weight gain, as the evidence is stronger for long-term consequences of maternal undernutrition ⁷⁴. Also, the weight gain recommendations are based solely on observational studies, and may therefore not be causally linked with the studied outcomes ⁶⁸.

Table 4. Weight gain recommendations during pregnancy across pre-pregnancy BMI category according to the U.S. Institute of Medicine ⁶⁸.

Pre-pregnant BMI	Total weight gain range (kg)	Rates of weight gain in 2 nd and 3 rd trimester Mean (range) in kg/week
Underweight	12.5-18.0	0.51 (0.44-0.58)
Normal weight	11.5-16.0	0.42 (0.35-0.50)
Overweight	7.0-11.5	0.28 (0.23-0.33)
Obese	5.0-9.0	0.22 (0.17-0.27)
First trimester weight gain is estimated to 0.5-2.0 kg		

1.2.1 Gestational weight gain and ethnicity

The U.S. IOM recommendations are not ethnicity specific, despite considerable ethnic differences in percent fat mass and disease risk for the same BMI, as outlined previously. Studies exploring ethnic differences in gestational weight gain are scarce, and IOM therefore encourage research on gestational weight gain across ethnic groups ⁶⁸. We were only able to find two studies from Europe that reported weight gain across ethnic groups ⁶⁷. One study found a statistically lower weight gain in Asians and Africans compared to the Swiss host population ⁷⁵. The other study did not statistically test the ethnic differences in weight gain,

but there was a tendency towards a lower weight gain in Sri Lankan and African women compared to the Swiss host population ⁷⁶.

1.3 HYPERGLYCAEMIA

The blood glucose concentration needs to be high enough to secure energy to the brain, as it is the main fuel that can be utilized by the brain. In addition, the blood glucose needs to stay low enough to not damage tissues, as hyperglycaemia is known to cause damage to blood vessels. In the normoglycaemic state, insulin is produced and released in relation to the amount of glucose in the blood to optimize blood glucose concentration. Insulin works as a signal molecule, with several effects on metabolism and growth. The primary function of insulin is telling the tissues to transport glucose from the blood stream into the cells, so that the cells can either use the glucose as an immediate energy source or store it for later use ⁷⁷.

In the fasting state, normoglycaemia is ensured by gluconeogenesis or glycolysis. Gluconeogenesis is production of glucose from non-carbohydrate substrates such as pyruvate, lactate, glycerol or certain amino acids. Gluconeogenesis is primarily present in the liver, although some gluconeogenesis is also present in the kidneys. Glycogenolysis is breakdown of glucose stores (glycogen), which mainly appears in the liver. Presence of insulin suppresses gluconeogenesis and glycogenolysis ⁷⁷.

After ingestion of a meal with carbohydrates, absorption of glucose will lead to an increased glucose concentration in the blood. In muscle cells, insulin activates glucose transporters (GLUT 4) who are translocated to the cell membrane, leading to removal of glucose from the blood and into the muscle and adipose tissue. However, tissues do not always respond adequately to insulin, and reduction of metabolic effects of insulin is termed insulin resistance. Peripheral insulin resistance—when muscle cells, adipocytes or other peripheral cells are insulin resistant—mainly results in high postprandial glucose as the muscle cells are not able to remove glucose from the blood rapidly enough. Hepatic insulin resistance, on the other hand, results primarily in high fasting glucose as neither gluconeogenesis nor glycogenolysis is adequately suppressed by the presence of insulin. Usually beta-cells compensate for elevated insulin resistance by producing extra insulin. When the beta-cells are unable to compensate for the increased insulin resistance, it results in hyperglycaemia and eventually frank diabetes.

Insulin sensitivity is the reciprocal of insulin resistance and the two terms are often used interchangeably, although insulin resistance is thought to be the result of both reduced insulin sensitivity and reduced insulin response⁷⁸.

Genetics may play a role in both insulin resistance and beta cell function, although a majority of the candidate genes are related to insulin secretion than to insulin resistance⁷⁹. Insulin resistance is strongly associated with adiposity²², especially central adiposity⁵. Visceral fat and ectopic fat accumulation in the pancreas have also been proposed as possible mechanisms for reduced beta-cell function^{80,81}.

1.3.1 Hyperglycaemia in pregnancy

Pregnancy is accompanied by a number of metabolic and physiological changes to secure nutritional flow to the foetus for adequate growth and development⁸². Glucose crosses the placenta and is the preferred energy source for the foetus. Maternal insulin, on the other hand, does not cross the placenta.

In 12-14 weeks' gestation, the change in insulin resistance seems to be inversely related to the simultaneous accretion of fat mass⁸³. In the last third, however, insulin sensitivity is decreased with 50-60 %, independently of the insulin sensitivity before pregnancy⁸⁴⁻⁸⁶.

Despite the high increase in insulin resistance, which otherwise may result in hyperglycaemia, the blood glucose levels remain quite stable throughout pregnancy as the foetus utilizes a large part^{87,88}. However, the stable blood glucose levels throughout pregnancy also depend on the ability of pancreatic beta cells to compensate by increasing insulin secretion^{88,89}.

However, if the beta cells are unable to compensate for the increased insulin resistance, hyperglycaemia will ensue.

Pedersen⁹⁰ suggested already in 1952 that maternal hyperglycaemia transmits to the foetus and induce foetal hyperinsulinaemia that stimulates foetal growth and thus may lead to increased birth weight and excessive body fat in the offspring⁹⁰. In accordance with the Pedersen hypothesis, maternal hyperglycaemia is associated with increased foetal insulin⁹¹, and with increased birth weight⁹²⁻⁹⁴ and fat mass⁹⁵ in the offspring.

1.3.2 Gestational diabetes

GDM is associated with increased risk of later T2DM in the mother ^{18,96}. Maternal type 1 diabetes, T2DM and GDM have all been associated with increased risk of future obesity and T2DM in the offspring ^{15,97}. GDM is associated with high birth weight, cord blood c-peptide level, caesarean delivery and neonatal hypoglycaemia ¹⁷, as well as metabolic syndrome in childhood for the offspring ⁹⁸. In Europe, the prevalence of GDM varies greatly depending on population studied, screening method performed and diagnostic criteria used ⁹⁹.

Diagnostic criteria

O'Sullivan was the first to introduce the screening glucose challenge test to detect GDM ¹⁰⁰, and O'Sullivan and Mahan ¹⁰¹ were the first to develop pregnancy specific criteria that differed from the T2DM criteria used outside pregnancy. The O'Sullivan and Mahan criteria were later modified to compensate for advancing laboratory techniques first by the National Diabetes Data Group ¹⁰² and later by Carpenter and Coustan ¹⁰³. The Carpenter-Coustan criteria have been used extensively since, especially in the U.S.A. The American Diabetes Association (ADA) has relied on the Carpenter-Coustan criteria, but introduced the option of performing a 75 g OGTT over 2 hours instead of a 100 g OGTT over 3 hours in 2003 ¹⁰⁴. The criteria based on the O'Sullivan and Mahan criteria are all based on a two-step screening; all women undergo a non-fasting 1-hour 50 g glucose load test. If 1-hour glucose levels are above 7.8 mmol/l, a 100 g oral glucose tolerance test (OGTT) is performed.

The WHO operated with the same criteria for GDM as for T2DM up to 1999, when the 2-hour cut-off value for impaired glucose tolerance (≥ 7.8 mmol/l) was included in the GDM criteria ¹⁰⁵. The WHO 1999 criteria have since then been used by several European countries, including Norway ⁹⁹.

The use of several different criteria across countries has reduced the comparability across studies and thereby slowing progression of the research. The HAPO study was set up to meet the need of international consensus on diagnostic criteria for GDM ¹⁰⁶ and was a large multicentre cohort of more than 25 000 healthy pregnant women ¹⁷. Instead of preventing future T2DM risk in the mothers, which was the aim of the O'Sullivan and Mahan criteria ¹⁰¹, the HAPO study aimed to find thresholds to reduce the frequency of the primary outcomes; high birth weight, caesarean section, hypoglycaemia in the newborn and high c-peptide levels

in the newborn. The HAPO study found a continuous relationship between maternal glucose levels and all primary outcomes ¹⁷ suggesting that hyperglycaemia may be detrimental at a lower level than previously thought. The International Association of the Diabetes and Pregnancy Study Groups (IADPSG)—an umbrella organization to facilitate collaboration between research groups with a focus on diabetes in pregnancy—sponsored a workshop in 2008 gathering 225 researchers from 40 countries to review findings from the HAPO study and to discuss GDM criteria, which resulted in a proposition for new GDM criteria ¹⁰⁷ (Table 5).

Table 5. Overview of different criteria used to diagnose GDM.

75 g OGTT			75 or 100g OGTT	100 g OGTT		
	WHO 1999	IADPSG 2010	ADA 2003	O'Sullivan & Mahan 1964	National Diabetes Data Group 1979	Carpenter & Coustan 1982
Screening method	At risk individuals	One-step universal screening	Two-step universal screening	Two-step universal screening	Two-step universal screening	Two-step universal screening
Fasting	Venous plasma (mmol/l)	Venous plasma (mmol/l)	Venous plasma (mmol/l)	Venous whole blood (mmol/l)	Venous plasma (mmol/l)	Venous plasma (mmol/l)
	≥ 7.0	≥ 5.1	≥ 5.3	≥ 5.0	≥ 5.8	≥ 5.3
	1 h	≥ 10.0	≥ 10.0	≥ 9.1	≥ 10.6	≥ 10.0
	2 h	≥ 8.5	≥ 8.6	≥ 8.0	≥ 9.2	≥ 8.6
	3 h		≥ 7.8	≥ 6.9	≥ 8.1	≥ 7.8
Abnormal values needed	1	1	2	2	2	2
Additional comments		Adopted by ADA 2012 and WHO 2013	3 h cut-off only applies if 100 g OGTT is performed			

OGTT = Oral glucose tolerance test; h = hour; WHO = World Health Organization; IADPSG = International Association of Diabetes and Pregnancy Study Groups; ADA = American Diabetes Association. Table adapted from Buckley et al. ⁹⁹.

If the GDM criteria developed by the IADPSG ¹⁰⁷ are used, the prevalence tends to increase compared to formerly used criteria, leading to concerns regarding labelling a large fraction of pregnant women as ill ¹⁰⁸. The IADPSG criteria were adopted by the ADA in 2011—although ADA reintroduced the Carpenter and Coustan based criteria in 2014 due to lack of evidence ¹⁰⁹—and by the WHO in 2013 ¹¹⁰. However, the WHO does not explicitly recommend universal screening, and do not demand collection of 1-hour glucose values.

1.3.3 Gestational diabetes and ethnicity

Studies from Europe find a higher risk of GDM in several ethnic minorities with non-western origin compared to the host populations⁶⁷. South Asians unambiguously have a higher risk of GDM, while the GDM risk seem to vary within other ethnic groups in studies from Europe⁶⁷. The prevalence of GDM in the STORK Groruddalen study by the WHO 2013 criteria (based on fasting and 2-hour glucose, 1-hour glucose not available) was 31.5 %, whereof 24.0 % among Western Europeans and 36.8 % among non-western ethnic minority women¹¹¹. South Asians had the highest prevalence, with a 1.8 fold higher risk of GDM compared to Western Europeans¹¹¹. South Asians have been found to have high degree of insulin resistance, which is thought to contribute greatly to their higher diabetes risk¹¹². Findings from the U.S.A. indicate that some ethnic groups may have a higher risk of recurrence of GDM in subsequent pregnancies than “non-Hispanic White”¹¹³, while ethnic differences in recurrence rates of GDM in Europe does not seem to have been studied.

1.4 DYSLIPIDAEMIA

Dyslipidaemia refers to abnormal amounts or composition of blood lipids, such as cholesterol and fatty acids. High serum levels of triglycerides and low density lipoprotein (LDL)-cholesterol and low levels of serum high density lipoprotein (HDL)-cholesterol have been associated with insulin resistance, the metabolic syndrome, atherosclerosis and increased risk of cardiovascular disease¹¹⁴⁻¹¹⁶. Lately, excess energy and high circulating lipid levels have been associated with accumulation of ectopic fat, which in turn is associated with reduced function of the affected organ and metabolic disturbances¹¹⁷.

Insulin resistance is closely associated with dyslipidaemia^{22,118}, especially hypertriglyceridaemia, as insulin resistance leads to increased flux of non-esterified fatty acids (NEFA) which results in increased production and decreased clearance of the triglyceride-rich very-low density lipoprotein (VLDL) particles¹¹⁹. Low HDL-cholesterol and small dense LDL-cholesterol are also highly associated with insulin resistance and T2DM, although the exact mechanisms are not well understood¹¹⁹. There are some indications that dyslipidaemia could be a causal factor for insulin resistance¹²⁰. Obesity, and especially central obesity, is also highly associated with dyslipidaemia, although this relationship could be mediated by insulin resistance²⁷.

1.4.1 Dyslipidaemia in pregnancy

The concentration of maternal serum lipids changes during the normal pregnancy, probably mostly due to increased insulin resistance and hormonal changes⁸⁸. Although the placental transfer of most lipids is low, except for essential fatty acids and cholesterol, lipids play an important role in indirect provision of nutritional fuels to the foetus, such as ketone bodies which pass the placenta⁸⁷.

In early pregnancy, maternal body fat accumulation is increased, while during the last third of pregnancy there is a breakdown of maternal fat depots⁸⁷. The increased insulin resistance, in combination with several other changes in pregnancy, contribute to the increased lipolytic activity in maternal fat depots. The breakdown of maternal fat depots results in the release of NEFAs and glycerol which are transported to the liver. In the liver, glycerol may be used as a substrate for gluconeogenesis, and NEFA for synthesis of ketone bodies, or to form VLDL-cholesterol to transport triglycerides to peripheral tissue for energy production or energy storage^{77,87}. In late pregnancy, the activity of lipoprotein lipase is reduced due to the increased insulin resistance⁸⁸, resulting in hypertriglyceridemia as the VLDL is not as effectively removed from the blood^{87,88}.

In women with GDM, the dyslipidaemia is further exacerbated as they often start their pregnancy with a higher degree of insulin resistance compared to healthy pregnant women. Women with GDM have elevated serum triglycerides¹²¹. Studies have found associations between maternal lipids during pregnancy and foetal growth, especially maternal triglycerides and HDL-cholesterol¹²²⁻¹²⁷. One study recently found that total cholesterol was of similar importance as maternal glucose for predicting birth weight¹²⁸. These results may indicate that increased maternal lipid levels provide the foetus with increased amounts of energy.

1.4.2 Maternal dyslipidaemia and ethnicity

Ethnic differences in maternal serum lipids are scarcely reported. In the Amsterdam Born Children and their Development study, lipid levels varied across ethnic minority groups, with the most detrimental lipid profile in Surinam-Hindustani and Turkish women¹²⁹. However, the adverse lipid profiles were largely explained by BMI¹²⁹. Another study in pregnant women found that Asian Indians had lower HDL-cholesterol and higher serum triglycerides compared to ethnic European women¹³⁰. These findings in pregnant women are supported by

results from non-pregnant, showing that South Asians have low HDL cholesterol and high triglycerides ^{131,132}; Turkish women have high levels of serum triglycerides ¹³³, while black Caribbean and black African seem to have more favourable lipid profiles ¹³⁴.

1.5 MECHANISMS LINKING ADIPOSITY, HYPERGLYCAEMIA AND DYSLIPIDAEMIA

Adiposity, especially central adiposity, is strongly associated with insulin resistance and dyslipidaemia. The association between central adiposity and metabolic disturbances is not necessarily causal, but could simply be a result of being coincidentally associated with the same causal factor ¹³⁵. There are, however, indications of reversibility, with reduction of visceral fat causing greater insulin sensitivity or improved beta cell function, suggesting a possible causal role of central adiposity. Lim and co-workers ¹³⁶ showed that 8 weeks on a very low calorie diet resulted in weight loss, decreased pancreatic and hepatic triglyceride content, and improved insulin sensitivity and beta cell function, resulting in normalization of fasting glucose and HbA1c levels. Conversely, eight weeks of overfeeding led to weight gain, decreased insulin sensitivity and increased levels of intrahepatic lipids ¹³⁷. Barzilai and co-workers ¹³⁸ found a marked improvement of insulin sensitivity and reduction in hepatic glucose production after visceral fat was removed surgically, although this experiment was performed in rats and thereby not necessarily transferable to humans. Further support of the importance of fat localisation in relation to diabetes risk is the finding that insulin sensitizing agents such as pioglitazone, which lead to a shift in body composition from central fat distribution to more peripheral fat storage even though the total fat mass is often increased, has shown to reduce insulin resistance ¹³⁹.

Dyslipidaemia has also been proposed as a causal factor for insulin resistance, as high circulating lipid levels are thought to cause ectopic lipid accumulation ¹¹⁷. A mendelian randomization study did not find any effect of genetically raised triglyceride levels on risk of T2DM ¹⁴⁰. Also, LDL-cholesterol has been excluded as a potential causal factor for insulin resistance ¹²⁰. However, there are studies indicating that lipoprotein lipase function may be important in impaired insulin sensitivity ¹²⁰.

There is increasing consensus that ectopic fat accumulation in muscle and liver is important in the development of insulin resistance in humans ¹¹⁷. Evidence is also emerging, indicating that ectopic fat accumulation in the pancreas may be responsible, at least partially, for the beta cell dysfunction seen in diabetes ^{80,81}. In concordance with these findings are also the previously mentioned findings that substantial weight loss led to decreased pancreatic and hepatic fat, normalized beta cell function and reversal of T2DM ¹³⁶. Another study showed that lipid content in the pancreas was higher in T2DM cases than in controls ⁸⁰.

1.6 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

The Developmental Origins of Health and Disease (DOHaD) concept represents the idea that poor conditions in early life increase the risk of non-communicable disease in adult life, such as cardiovascular disease, T2DM, obesity and other metabolic disorders ¹⁴¹. Anders Forsdahl ¹⁴² found that cohorts born in years with high infant mortality were succeeded by higher mortality from cardiovascular risk 40-60 years later than cohorts born in years with lower infant mortality, and concluded that poor living conditions in early life followed by abundance could be a risk factor for cardiovascular events ¹⁴². Hales and Barker with co-workers proposed their theory of the thrifty phenotype in the 1990s, suggesting that fetuses exposed to undernutrition are programmed in utero to cope with harsh living conditions in later life. They found that low birth weight was highly associated with several diseases in adulthood, including T2DM ^{143,144}. Although Hales' and Barker's hypothesis that low birth weight increased risk of T2DM led to controversy when they presented it, their hypothesis was later supported by studies in twins, showing that birth weight was lower in twins who developed T2DM in adulthood compared to their non-diabetic mono-zygotic twin ¹⁴⁵. The detrimental effects of being born small for gestational age are now well established.

The DOHaD concept is largely based on the ideas of Hales and Barker, and was further supported by findings from the Dutch Hunger Winter study ¹⁴⁶. The Dutch Hunger Winter study showed that maternal undernutrition during pregnancy affected offspring's adult health independently of social class and other potential confounding factors, and that the timing of undernutrition was important ¹⁴⁷.

1.6.1 The developmental overnutrition hypothesis

Historically, there has been a major focus on the relationship between maternal undernutrition, low birth weight and chronic disease in adult life. However, during the last decades, with the evolving obesity epidemic, concerns about maternal overnutrition have emerged. The developmental overnutrition hypothesis suggests that overnutrition in utero—driven by obesity, excessive weight gain or diabetes in the mother—lead to permanent metabolic changes in the foetus which, in turn, will increase the risk of adiposity and diabetes in later life ⁹¹. However, whether these associations are due to in utero programming of the foetus or shared genes and lifestyle between the mother and her offspring, are debated.

Although shared genes and lifestyle are thought to play a major part in the risk of offspring adiposity, studies in siblings suggest a strong intra uterine effect of diabetes. A study that explored the effect of maternal diabetes and BMI in siblings born before and after onset of diabetes in the mother, showed that intrauterine exposure to maternal diabetes substantially increased the offspring's BMI (BMI was 2.6 (95 % confidence interval (CI) 0.9-4.3) kg/m² higher in exposed siblings) and the risk of T2DM (odds ratio (OR) (95 % CI) 3.7 (1.3-11.3) for exposed siblings)¹⁶. However, they found no differential risk in siblings born before or after onset of diabetes in their father ¹⁶. A study of female Pima Indians with a mutation resulting in maturity-onset diabetes of the young showed that diabetes developed 12 years earlier in offspring of whose mothers had already developed diabetes prior to pregnancy compared to offspring whose mothers had not yet developed diabetes ($P < 0.0001$) ¹⁴. They found no difference in age at diagnosis when the father was diagnosed at a young age ¹⁴. Similarly, a study in siblings found that excessive gestational weight gain during pregnancy contributed to later offspring obesity in overweight and obese, but not in normal weight mothers ¹⁴⁸. However, a large part of the associations between gestational weight gain and later offspring BMI were explained by shared familial characteristics. In relation to pre-existing obesity, a study that followed siblings born before or after large weight loss after obesity surgery found that maternal surgery substantially reduced the prevalence of obesity in the 2-18 year old offspring ¹⁴⁹.

1.7 NEONATAL ANTHROPOMETRY

Big babies are associated with increased risk of complications such as caesarean section, shoulder dystocia, asphyxia and neonatal hypoglycaemia¹⁵⁰. High birth weight has been associated with long-term risk for childhood and adult overweight and obesity^{151,152}, as well as T2DM in later life¹⁵³. Gestational weight gain seems to play an independent role in the development of high birth weight and adiposity in the neonates⁶⁸.

Although easy to measure, birth weight is generally considered a rough indicator of foetal growth, as the differences in birth weight may be attributed both to differences in fat and lean mass¹⁵⁴. Hence, neonatal fat mass is considered a more sensitive marker of the foetal environment and a more specific outcome measure of foetal growth than birth weight^{155,156}. Although neonatal fat mass has been suggested as an early indicator of childhood obesity, the long-term health impact of neonatal fat mass seems to be scarcely studied.

1.7.1 Neonatal anthropometry and ethnicity

The optimal birth weight may differ across different populations, and studies find distinct differences in birth weight across ethnic groups, with offspring of women originating from low income countries having lower birth weight than European host populations⁶⁷. Offspring of Africans tend to have a relatively high birth weight, while offspring of South Asians tend to have a low birth weight compared to offspring of Europeans⁶⁷. However, despite lower birth weight, babies born in India seem to preserve their subcutaneous fat mass compared to babies born in England, referred to as the thin-fat phenotype¹⁵⁷.

In the STORK Groruddalen Study, offspring of women from Pakistan, Sri Lanka/India or East Asia had lower birth weight than Western Europeans¹⁵⁸, while there were no difference between Western Europeans and offspring of women from the Middle East, sub-Saharan Africa or Eastern Europe. However, among the offspring with low birth weights, Pakistani offspring were the only group who had less subcutaneous fat than Western Europeans, while Sri Lankans/Indians and East Asians had similar amounts of subcutaneous fat¹⁵⁸.

Subcutaneous fat in the newborn may be a better indicator of the foetal environment than birth weight in a multi-ethnic population, and possibly a better predictor for future health of the offspring.

2 AIMS

The overall aims of this thesis were to increase the understanding of how maternal adiposity is related to GDM, and how maternal adiposity, hyperglycaemia and dyslipidaemia influence the newborn offspring's birth weight and amount of subcutaneous fat.

The specific objectives were to:

- explore the effect of mid-gestational weight gain and gain of total fat, central fat and subcutaneous fat on the risk of GDM (Paper 1)
- explore differences between Europeans and South Asians in BMI, subcutaneous fat and s-leptin levels during and after pregnancy (Paper 2)
- explore the effect of BMI, subcutaneous fat and s-leptin levels in early pregnancy on the risk of GDM (Paper 2)
- explore whether the effects of maternal glucose and lipid levels on offspring's birth weight and subcutaneous fat were independent of early pregnancy BMI and mid-gestational weight gain (Paper 3)

3 METHODS

3.1 STUDY DESIGN AND SUBJECTS

The methods of the STORK Groruddalen study have been described in detail previously ¹⁵⁹⁻¹⁶¹. The STORK Groruddalen study is a prospective cohort study of pregnant women living in Groruddalen, a multi-ethnic area of Oslo. Inclusion was conducted during 2008-2010. Groruddalen cover affluent as well as more deprived residential areas. A local information campaign was set up prior to the recruitment of pregnant women, and all general practitioners in the study area were asked to refer pregnant women as early as possible to the child health clinics. Women were recruited by midwives in early pregnancy at three child health clinics, covering three out of the four districts in Groruddalen. Women were eligible if they: 1) lived in the study districts; 2) planned to give birth at one of two study hospitals; 3) were in < 20 week's gestation; 4) could communicate in Norwegian or any of the eight translated languages; 5) were able to give an informed consent. Women with pre-existing diabetes or in need of intensive hospital follow-up during pregnancy were excluded.

To facilitate the inclusion of Pakistani and Somali women, the inclusion criteria was slightly changed 6 months after study start to allow for inclusion of these groups if < 25 weeks' gestation ¹⁶¹.

The attendance of the STORK Groruddalen cohort is presented in Figure 3 and has been described in detail elsewhere ¹⁵⁹. The participation rate was 74 % (Figure 3), and varied from 82 % in Europeans, 71 % in Asians, 65 in Middle Easterners to 64 % in Africans ¹⁵⁹. Age did not differ between the 823 who participated and the 291 who chose not to participate ¹⁵⁹. South Asians who did not participate were more parous than those who participated, while the rates for parity were similar within the remaining ethnic groups ¹⁵⁹. The study cohort was found to be fairly representative for the main ethnic groups ¹¹¹, and there were no ethnic differences in reasons for exclusion ^{111,159}.

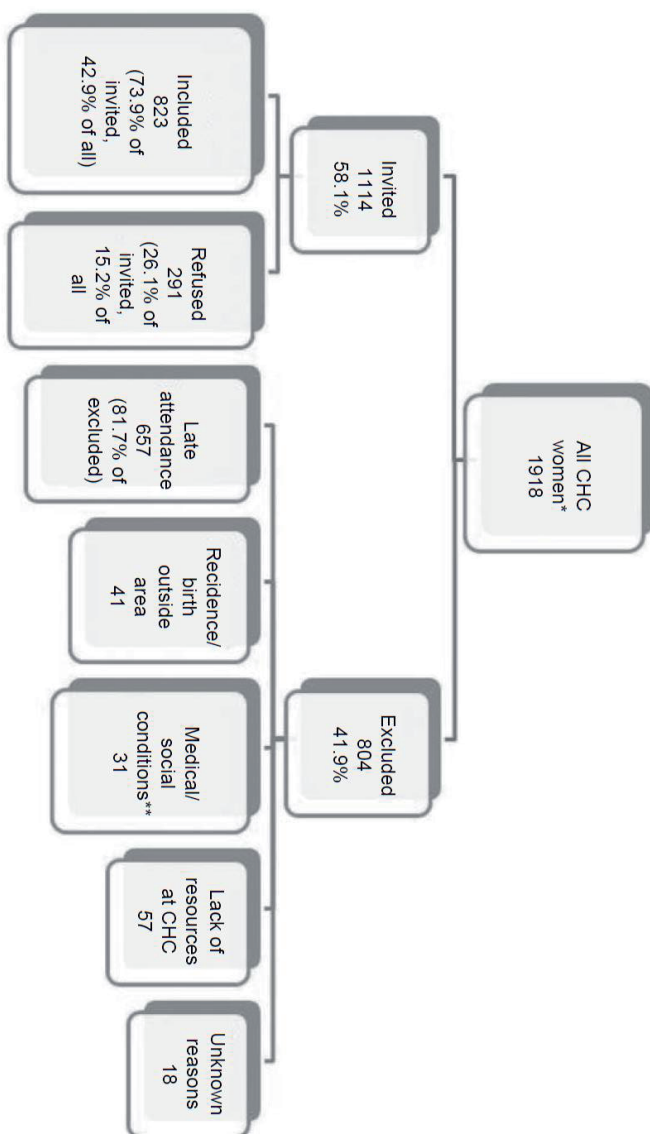


Figure 1. Attendance at the CHC from 6 May 2008 to 15 May 2010, those invited to the study, those who refused participation and those excluded categorised by reasons for not being invited. *represents number of pregnancies, of women attending the CHC in this period, 42 women represented with two pregnancies (1876 unique women). Approximately 50% of those with two pregnancies were included in their second pregnancy. **includes nine women excluded in one pregnancy as they were already included in the study.

Figure 3. Attendance in the STORK Groruddalen cohort.
Reprint from Jenum et al. 2010 ¹⁵⁹ with permission from SAGE Publications.

The flow of the cohort is described in Papers 1-3 and in Figure 4.

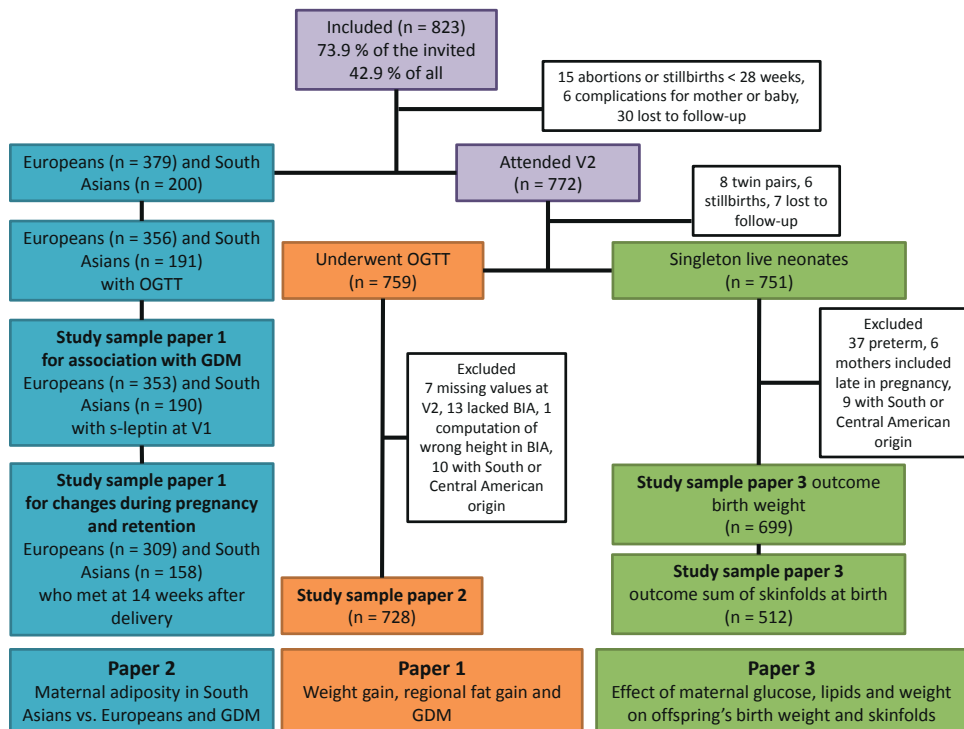


Figure 4. Study samples for papers 1-3.

V2—Visit 2, OGTT—Oral glucose tolerance test, BIA—bioelectrical impedance analysis.

3.2 ETHICS

The study was approved by the Regional Ethics Committee. Written consents were obtained from each woman on behalf of herself and her offspring. The storage of biological material was approved by the Regional Ethics Committee and the Norwegian Directorate of Health. Data was stored in agreement with the standards of the Norwegian Data Inspectorate and handled anonymously. The study was conducted in agreement with the Helsinki declaration.

3.3 DATA COLLECTION

Women were included at (mean \pm standard deviation (SD)) 15 ± 3 weeks' gestation (Visit 1), re-examined at 28 ± 1 weeks' gestation (Visit 2) and 14 ± 3 weeks after delivery (Visit 3) (Table 6). All information material and questionnaires were translated to Arabic, English, Sorani, Somali, Tamil, Turkish, Urdu and Vietnamese and quality controlled by bilingual health professionals. Professional interpreters were used when necessary (for 22 % of the women ¹⁶¹).

Table 6. Overview of collected data used in this thesis.

	Pre-pregnancy	Visit 1 (week <20)	Visit 2 (week 21-29)	Delivery (0-72 hours after delivery)	Visit 3 (10-14 weeks after delivery)	Used in Paper
Maternal						
Ethnicity, age, parity, family history of diabetes ¹ , smoking ²		X				1, 2, 3
Height		X				1, 2, 3
Weight	Self-reported at Visit 1	X	X		X	1, 2, 3
Bioelectrical impedance analysis		X	X		X	1
Skinfolds		X	X		X	1, 2
Biological markers		X	X		X	
Fasting glucose		X ³	X ^{3,4}		X ³	1, 2, 3
2-hour glucose			X ^{3,4}			1, 2, 3
C-peptide		X	X		X	1, 2
Leptin		X	X		X	2
Insulin		X	X		X	2
HbA1c		X	X		X	2
Total cholesterol		X	X		X	3
HDL-cholesterol		X	X		X	3
LDL-cholesterol		X	X		X	3
Triglycerides		X	X		X	3
Neonatal						
Gestational age ⁵						3
Weight				X		3
Skinfolds				X		3
Sex				X		3

¹ Used only in paper 2.

² Used only in paper 3

³ Glucose measured in serum (Vitros).

⁴ Glucose measured in full blood on site (Hemocue)

⁵ Based on mothers last menstrual period unless large variation from age estimated with routine ultrasound at 17-20 weeks' gestation.

3.3.1 Ethnicity

In this thesis, ethnicity was defined as country of birth or participant's mother's country of birth if the participant's mother was born outside of Europe or North America, in correspondence with suggestions from Senior and Bhopal ¹⁶². In the three papers of this thesis, the terms ethnicity, ethnic origin etc. are used inter-changeably, but all refer to the above definition.

The STORK Groruddalen study comprises women born in 65 different countries. To assure statistical power, countries of birth were merged into ethnic groups, while trying to retain certain homogeneity in the groups (Table 7). Three women from North America (whereof two had ethnic Norwegian ancestry) were categorized into the ethnic group "Europe", as they were considered to be quite homogenous. Somalia was categorized together with 5 women from other countries of the Horn of Africa, 12 women from other Sub-Saharan countries and with 5 women from Nigeria into the ethnic group "South and Central Africa".

Table 7. Overview of the classification of ethnic groups used in this thesis.

	Europe	South Asia	East Asia	Middle East	South and Central Africa	Excluded	Total
Norway	313						
Eastern Europe	43						
Other Scandinavia	13						
Other Western European countries	7						
North America	3						
Pakistan		126					
Sri Lanka		61					
Other South Asian countries		13					
Vietnam			18				
The Philippines			13				
Other East Asian countries			13				
Iraq				38			
Turkey				28			
Morocco				27			
Afghanistan				13			
Other Near or Greater Middle Eastern countries				20			
Somalia					40		
Other African countries South of Sahara					22		
South or Central America						12	
	379	200	44	126	62	12	823

Only 52 women (6.3 %) were second generation immigrants from outside Europe or North America, and thereby classified into their mother's country of birth. The majority of these originated from Pakistan (n = 40).

In papers 1 and 3 all ethnic groups, except women from South or Central America, were included, while in paper 2, only Europeans and South Asians were included, (Table 7). In all three papers, we excluded women from South or Central America due to low numbers and heterogeneity of ethnic origin.

3.3.2 Questionnaire data

Questionnaire data used in this thesis were collected at Visit 1. All questionnaires were interviewer-administered by midwives and study personnel. *Parity* was defined as previous viable pregnancies and used as a dichotomized variable; parous or nulliparous. Maternal *age* was calculated from participant's date of birth. *Weeks' gestation* was based on first day of the last menstrual period. Due to low numbers of smokers in the cohort, we merged two questions regarding smoking; 1) smoked within three months prior to conception, and 2) smoked during pregnancy, and dichotomized into non-smokers versus occasional or regular smokers. The women were asked about their *pre-pregnancy body weight* shortly after being weighed at Visit 1.

3.3.3 Maternal anthropometrics

Maternal height was measured at visit 1 by a fixed stadiometer, calibrated before study start and thereafter biannually. Pre-pregnancy body weight was self-reported at visit 1 and weight was measured at Visit 1, Visit 2 and Visit 3, to the nearest 0.1 kg by a digital scale, calibrated before study start and thereafter biannually (Tanita-BC 418 MA, Tanita Corporation, Tokyo, Japan). BMI was calculated as the weight in kg divided by the height in meters squared.

Total body fat and central fat (referred to as truncal fat in paper 1) was measured at Visit 1, and Visit 2, with a BIA scale (Tanita-BC 418 MA). The use of Tanita-BC 418 MA has been validated in humans¹⁶³ and is not thought to result in systematic bias when estimating fat mass in pregnancy³³. As the accuracy varies across ethnic groups, as previously discussed, we used the intra-individual change to avoid potential bias across ethnic groups.

Skinfolds were measured twice at Visit 1, Visit 2 and Visit 3, to the nearest 1 mm with a caliper (Holtain T/W Skinfold Caliper, Holtain Ltd., Crymch, UK). The mean value of the two measurements was subsequently used. The skinfolds were measured at the triceps, subscapular and suprailiac sites. In paper 1, we used the mean of all three skinfold sites as a variable. In paper 2, we summarized all three sites, and used sum of skinfolds as a variable. Five raters measured maternal skinfolds after receiving extensive training. Inter-rater variability, expressed as % Technical Error of Measurement (%TEM)³⁸ was assessed twice a year. Inter-rater variability ranged from 5 to 21 % between study personnel. Intra-rater variability was less than 5 % for all measurements¹⁵⁸.

We used the measured weight gain between Visit 1 and Visit 2 divided by number of weeks between the two measurements to explore mid-gestational weight gain. We used IOMs recommended range of weight gain per week in second and third trimester for specific BMI categories⁶⁸ to classify women into having inadequate, adequate or excessive weight gain.

3.3.4 Laboratory methods

Fasting venous samples with serum were collected at Visit 1, Visit 2 and Visit 3. The samples were put on ice and daily shipped to the laboratory at the Department of Multidisciplinary Laboratory Medicine and Medical Biochemistry, Akershus University Hospital. Fasting glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were measured using a colorimetric method (Vitros 5.1 FS, Ortho Clinical Diagnostics)^{111,164}. Fasting C-peptide and insulin were measured at the Hormone Laboratory, Oslo University Hospital, by non-competitive immunofluorometric assays (DELFI, PerkinElmer Life Sciences, Wallac Oy, Turku, Finland). Homeostasis model assessment of insulin resistance (HOMA-IR) was estimated by the Oxford University HOMA Calculator 2.2 using C-peptide. HbA1c was analyzed in full blood with HPLC (Tosoh G8). Fasting venous samples of serum was biobanked and stored at -80°C prior to analysis of leptin levels. S-leptin levels were analysed by HADCYMAG-61K based on the Luminex® xMAP® technology (Milliplex® Map, Millipore Corporation, Billerica, MA, U.S.A.) at the Hormone Laboratory, Oslo University Hospital, Oslo.

At Visit 2, fasting and 2-hour glucose after a 75 g OGTT, were analysed both by using a patient near “Point of Care” method (HemoCue, Angelholm, Sweden) and by using the previously described method (Vitros).

Women were diagnosed with GDM at Visit 2 according to the WHO 1999 criteria (fasting glucose ≥ 7.0 mmol/l or 2-hour glucose ≥ 7.8 mmol/l). Women diagnosed with GDM by the WHO 1999 criteria received lifestyle advice and were referred to their General Practitioner for follow-up if 2-hour glucose was <9.0 mmol/l or to hospital care if 2-hour glucose was ≥ 9.0 mmol/l ¹¹¹.

In this thesis, GDM was defined by the WHO 2013 criteria (fasting glucose ≥ 5.1 mmol/l and 2-hour glucose ≥ 8.5 mmol/l) at Visit 2, due to the emergence of new criteria.

3.3.5 Neonatal characteristics

Gestational age was calculated from the first day of the woman’s last menstrual period (LMP) and term was calculated as date of LMP +282 days. If the LMP date was missing or differed ≥ 14 days from the term estimated by routine ultrasound in week 17-20, we used the ultrasound term ($n= 24$ (3.4%)) ¹⁵⁸. The outcome birth weight was measured with electronic scales immediately after birth ¹⁵⁹.

Neonatal skinfolds at subscapular, suprailiac, thigh and triceps sites were measured twice, to the nearest 0.2 mm, with a skinfold caliper (Holtain T/W Skinfold Caliper, Holtain Ltd., Crymych, UK) within 72 hours after birth. The mean value of the two measurements was subsequently used.

The sum of skinfolds was calculated by summarizing the four skinfold sites. Two raters measured neonatal skinfolds after receiving extensive training. Inter-rater variability (measured as % Technical Error of Measurement) for the skinfold measurements ranged from 8-13%, while the intra-rater variability varied from 2.9 % in the thigh skinfold to 4.3 % in the suprailiac skinfold ¹⁵⁸.

3.4 STATISTICS

All statistical analyses were performed by me, using IBM SPSS statistics 19-22 (Chicago, IL, USA). Data are presented as mean and SD if normally distributed, median and inter-quartile range if non-normally distributed and number and percent if categorical. Effect estimates were presented with 95 % confidence intervals. $P < 0.05$ was considered statistically significant. Non-normally distributed variables were transformed as appropriate to attain normal distribution prior to performing parametric statistical tests. We performed logistic regression analyses in papers 1 and 2, multivariate general linear models in paper 2 and linear regression in paper 3. In papers 1 and 3, we used the `lincom` command in StataIC 12 (StataCorp LP, College station, TX, USA) to calculate sole and combined risks based on the respective regression model.

To explore potential bias in the-self-reported pre-pregnancy weight, we used general linear models to explore differences between self-reported pre-pregnancy weight and measured weight at Visit 1, across BMI and ethnicity. In paper 3, we used analysis of variance to explore interference between quartiles of triglyceride levels and glucose measured with HemoCue to decide on whether to use glucose variables measured with Vitros or HemoCue in the paper.

3.4.1 *Standardization of explanatory variables*

In paper 2 and 3, we standardized the explanatory variables in order to easier compare their effect on the outcome against each other. The Z-score standardize continuous variables that are normally distributed by giving every observation a standardized value based on its number of SDs from the sample mean (Individual observation – sample mean / sample SD). E.g. if a participant has a BMI of 40, the sample mean BMI is 25.3 and the SD is 4.9, the participant's z-score will be $(40-25.3) / 4.9 = 3$, giving a standardized BMI of 3 SDs above the mean.

3.4.2 *Adjustments for covariates*

In all three papers, we adjusted for selected covariates that differed across the ethnic groups. Weeks' gestation at inclusion was adjusted for in all analyses, as it varied slightly between

ethnic groups. Age and parity were adjusted for in all analyses as they varied across ethnic groups, and as they have been associated with both gestational weight gain ⁶⁸ and GDM ¹⁶⁵.

3.4.3 Interactions

When exploring relationships across groups it is important to check whether the risk factors relate to the outcome similarly in the different groups. Relationships between risk factors and outcomes may vary across ethnic groups and relationships may differ when certain variables are taken into account. In all three papers we explored interactions between ethnicity and the explanatory variables.

3.4.4 Statistical power considerations

Power calculations were performed prior to study start to secure a large enough sample size to detect ethnic differences in the prevalence of GDM defined by the WHO 1999 criteria ¹⁵⁹. The expected prevalence of GDM was 5 % in Western women, 20 % in South Asians and 10 % in the remaining ethnic groups. Based on the ethnic composition attending the three child health clinics, the needed number of GDM cases was estimated to 100 women, and the sample size needed was estimated to 800 women ¹⁵⁹. The shift in GDM criteria during the study period resulted in more GDM cases than expected (99 according to the WHO 1999 criteria and 239 according to the WHO 2013 criteria) and thereby improved statistical power. Pre-study power calculations specific to this thesis were not performed, although we tried to preserve the statistical power by using continuous explanatory variables when appropriate. The statistical power was satisfactory for the three papers included in this thesis, especially for differences between the three largest ethnic groups.

4 RESULTS

4.1 PAPER I

Weight gain, total fat gain and regional fat gain during pregnancy and the association with gestational diabetes: a population-based cohort study.

In paper 1 we investigated the association between mid-gestational weight gain and gain of total fat, central fat and subcutaneous fat and GDM as defined by the WHO 2013 criteria.

We found that mean mid-gestational weight gain in our cohort was in the upper part of the intervals recommended by the IOM guidelines for weight gain during pregnancy. We did not find significant ethnic differences in weight gain, although differences in some of the other indicators of adiposity were observed. Women from Africa had significantly lower gain of total fat mass, central fat mass and mean skinfold thickness than Europeans, while East Asians had a higher gain of mean skinfolds than Europeans.

Mid-gestational weight gain and gain of total fat, subcutaneous fat and especially central fat were associated with increased risk of developing GDM.

4.2 PAPER II

Ethnic Differences in BMI, Subcutaneous Fat and Serum Leptin Levels During and After Pregnancy and Risk of Gestational Diabetes.

In paper 2 we explored differences between European and South Asian women in early pregnancy BMI, subcutaneous fat and s-leptin during and after pregnancy and their relationship with GDM as defined by the WHO 2013 criteria.

We found that South Asians had similar amounts of subcutaneous fat and higher s-leptin in early pregnancy (14 weeks' gestation) than Europeans, despite a lower BMI. South Asians, in comparison to Europeans, retained more weight and subcutaneous fat 14 weeks after delivery. Mean s-leptin levels decreased more in Europeans than in South Asians between 14 weeks' gestation and 14 weeks after delivery. At the examination 14 weeks after delivery, South Asians had more subcutaneous fat and higher levels of s-leptin than Europeans, and there

were no longer any difference in BMI between South Asians and Europeans. In early pregnancy (14 weeks' gestation) parous South Asians had more subcutaneous fat and s-leptin than parous Europeans, while we found no such ethnic difference in nulliparous. BMI, subcutaneous fat and s-leptin were all risk factors for GDM.

The higher weight and fat retention in South Asians may leave South Asians at a higher risk of GDM in future pregnancies.

4.3 PAPER III

Effects of early pregnancy BMI, mid-gestational weight gain, glucose and lipid levels in pregnancy on offspring's birth weight and subcutaneous fat. A population-based cohort study.

In paper 3 we explored whether the effects of maternal glucose and lipid levels on offspring's birth weight and neonatal subcutaneous fat were independent of early pregnancy BMI and gestational weight gain.

In this study we found maternal fasting glucose and HDL-cholesterol to be predictors of offspring's birth weight independently of mother's early pregnancy BMI and weight gain. Maternal fasting glucose and 2-h glucose were predictors of neonatal sum of skinfolds, also independently of BMI and weight gain. Still, weight gain was the strongest independent predictor of both outcomes. Fasting glucose and gestational weight gain were the only risk factor variables that were independent predictors for both birth weight and sum of skinfolds in the offspring. Furthermore, the effect of mother's early pregnancy BMI on birth weight was stronger in non-Europeans than in Europeans.

Mid-gestational weight gain may be more important than subsequent hyperglycaemia in the mother in predicting offspring's birth weight and subcutaneous fat.

5 DISCUSSION

5.1 METHODOLOGICAL CONSIDERATIONS

5.1.1 Inference of epidemiological studies

The inference of epidemiological studies is compromised by the presence of random errors, systematic errors and confounding. Observational studies will contain all three. Random errors can be reduced by a high degree of accuracy. Data collection will, however, always entail errors. Random errors make the results imprecise, create noise and make it harder to detect differences and thereby result in low reliability. Since the net effect of random errors will be close to zero, the results will reflect reality adequately, although it may reduce the statistical power and increase the risk of making statistical type 2 errors. Systematic errors, also known as bias, can be reduced by choosing gold standard methods. Presence of systematic errors may produce false mean values or effect estimates. If we are not aware of the systematic errors present in our material we may therefore draw the wrong conclusions. While random errors principally affect the statistical power and can be dealt with by increasing the sample size, bias will affect the internal validity of the data independently of the sample size. Control over potential bias is therefore crucial to secure internal validity and to interpret the results correctly.

A confounding variable is associated with both the dependent and the independent variable. Confounding in observational studies is often dealt with by adjusting for potential confounders. However, adjustment for confounders is not synonymous with ruling out their effect entirely, and sensitivity analyses should therefore be performed in addition to adjustments to reduce risk of residual confounding¹⁶⁶. In addition, observational studies cannot rule out the possibility of the effect being a result of unmeasured confounding. This is why randomized controlled studies are considered among the best to investigate causality, as they circumvent the problem of confounding. Due to random errors, bias and confounding within epidemiological studies they cannot single-handedly prove causal relationships, but causality may be assumed when the Bradford Hill criteria (Table 8) are satisfied¹⁶⁷.

Table 8. The Bradford Hill criteria ¹⁶⁷

1) Strength of the association
2) Consistency across several studies
3) Specificity of the association,
4) Temporal relationship
5) Biological gradient (i.e. dose-response)
6) Plausible mechanism
7) Coherence between observational studies and laboratory findings
8) Experiments supports the finding
9) Analogy – causality may be assumed if causation have been established for very similar cause and disease relationships

Random errors, bias and confounding will be discussed in the following sections.

5.1.2 Ethnicity

Bhopal defines ethnicity as a “multi-faceted quality that refers to the group to which people belong, as a result of certain shared characteristics, including geographical and ancestral origins, but particularly cultural traditions and languages” ¹⁶⁸. Ethnicity is complex, and is not easily described with a simple label. However, the exploration of why some ethnic groups have higher risk of disease has proven to be valuable in medical research ^{162,169}.

Ethnicity may be classified in several ways, and when used in epidemiological research, none are without limitations ¹⁶². How to classify ethnicity depends largely on the aim of the study ¹⁶², as geographical belonging may have a large impact on genetics, while belonging to a culture may have a large impact on dietary habits and other lifestyle choices. Also, large and heterogenic ethnic groups may hold large variations in country of birth, religion, dietary habits, physical activity and other variables that are related to health or disease ¹⁶². We defined ethnicity as country of birth or mother’s country of birth if born outside of Europe or North America, which is considered an objective but crude method for classification of ethnicity ¹⁶².

As a country may comprise heterogenous populations, the use of country of birth may mask some of the potential relationships with health and disease. Also, the father’s country of origin

may influence the offspring's health, which was not explored in this thesis. As this thesis aimed to explore differential relationships across ethnic groups between maternal factors, risk of GDM and the effect on birth weight and neonatal subcutaneous fat, we believe that the use of country of birth was the most appropriate way to define ethnicity in this thesis. Our aim was not to assess ethnic differences in dietary habits and physical activity, which is largely influenced by culture.

Merging of countries into ethnic groups may give heterogeneous groups, which in turn may lead to misleading results, such as neutralizing of effects. To diminish the risk of neutralizing effects, we examined the results using smaller and more homogeneous ethnic groups, but we cannot completely rule out this effect.

Health literacy is likely to differ across ethnicity, and although all questionnaires were interviewer-administered by trained study personnel, allowing possible misconceptions to be addressed immediately, we cannot rule out the possibility of ethnic groups understanding the questions differently.

5.1.3 Internal validity

Bias will compromise the internal validity, but if we are aware of potential bias it may help us to interpret the results more correctly.

Selection bias

The Groruddalen area of Oslo covers both affluent and more deprived areas¹⁵⁹, and the participating women had lower education compared to the general population of Oslo. The attendance rate was satisfying within all ethnic groups, but varied slightly across the ethnic groups. Ethnic Norwegian individuals with low socio-economic status are generally known to decline participation based on experience from several studies, while in the remaining ethnic groups we do not know who will decline to participate. However, all social leagues seem to be represented in all ethnic groups in our sample, which is reassuring.

In paper 1, we did not find any differences between those included and those excluded. In paper 2, we found that those who attended all three visits were more likely to be nulliparous and to have a lower BMI. However, since parous women were well represented, and all

categories of BMI were represented, we do not believe that this slight selection will affect the generalizability of our results considerably. In paper 3, a higher proportion of the excluded women were single and originated from South or Central Africa. This is probably because we excluded the six women who were included after 24 weeks' gestation, whereof 4 were from South or Central Africa. Women from Africa met for antenatal care later than women from the other ethnic groups. We do not believe that this slight selection will affect the generalizability of our results considerably.

Skinfold measurements were missing for 187 neonates, mostly due to delivery not being reported to study staff within 72 hours. However, the missing skinfold measurements seemed to be completely at random, as we did not find any difference in background variables between those with and without skinfold data.

Information bias

Maternal anthropometrics

Although the validity of BIA scales is known to vary across ethnic groups, we used fat mass and central fat mass estimated by a BIA scale. However, we used the intra-individual change, letting each participant be its own control, and bias is therefore thought to be minimal.

In paper 1 we adjusted for BMI, although it probably reflects a different degree of adiposity and diabetes risk across ethnic groups. However, we found no indications of this introducing bias when exploring interactions between Asians and ethnic-specific cut-off levels for BMI

The pre-pregnancy BMI was based on self-reported pre-pregnancy weight. Self-reported weight is generally highly subject to response bias. Obese women have been found to underestimate their body weight ¹⁷⁰. The validity of self-reported weight have also been found to vary across socio-economic groups ¹⁷¹ and across ethnicity ¹⁷². As the women were asked about their pre-pregnancy body weight shortly after being weighed, this may have reduced the risk of response bias. However, as the perceptions of what is a healthy body weight and the general attention given to body weight may differ across ethnic group and culture, the accuracy of the self-reported body weight may differ across ethnic groups in our data.

To explore potential bias in the self-reported body weight prior to pregnancy, we explored the difference between the self-reported pre-pregnancy body weight and the measured body weight at Visit 1 (Figure 5).

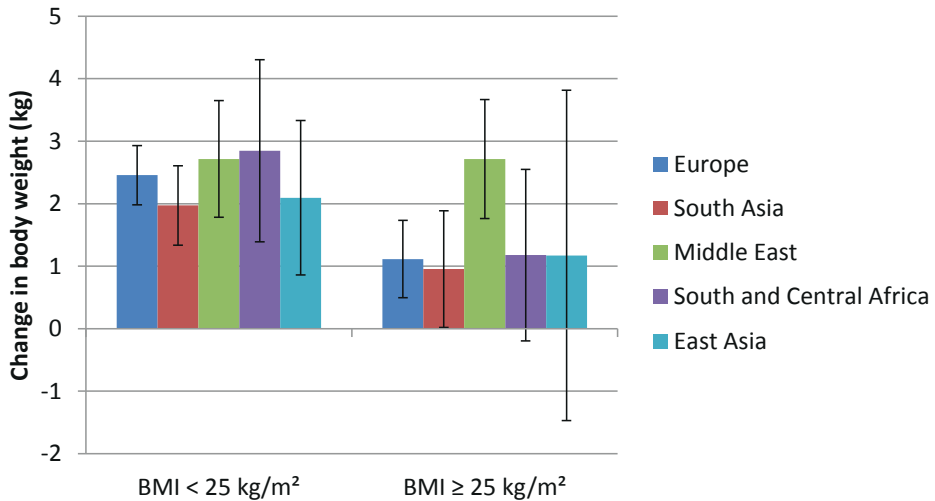


Figure 5. Change in body weight from pre-pregnancy (self-reported) to Visit 1 (measured) across ethnic groups and pre-pregnancy BMI.

We did not find any significant ethnic differences in weight change from self-reported pre-pregnancy up to 15 weeks' gestation at Visit 1. However, we found a tendency towards higher weight gain among Middle Easterners with BMI ≥ 25 kg/m² (P across ethnic groups 0.075 (Figure 5). We also found women with BMI ≥ 25 kg/m² to have a lower weight change from pre-pregnancy to Visit 1 than women with BMI less than 25 kg/m² (P = 0.016). This is, however, probably not a result of bias as overweight and obese women are expected to gain less weight during pregnancy than normal weight and underweight women. Also, our observation would imply that overweight and obese women overestimated their weight, while overweight and obese generally tend to underestimate their weight¹⁷⁰.

The maternal skinfold measurements had relatively large inter-rater variability, although this is not uncommon in epidemiological studies³⁸. The use of composite variables, such as sum of skinfolds, have been found to even out potential bias³⁸. Although there was no indication

of bias related to the large inter-rater variability ¹⁶¹, the high presence of random errors may weaken our power to detect potential associations with the outcomes tested.

Although use of MRI might have given more precise estimation of body fat and central fat, use of MRI probably would have compromised the participation rate, as the data collection would have demanded appearance at a hospital, instead of the community based approach, meeting the women where they receive conventional care.

Glucose and GDM

The measurement of glucose is not thought to differ in accuracy across ethnicity. Glucose levels measured in central laboratories are prone to give falsely low glucose levels due to pre-analytic glycolysis ¹⁷³. The ultimate goal of the STORK Groruddalen study was to estimate the prevalence of GDM in the population, and a falsely low content of glucose would give a falsely low prevalence of GDM. The GDM diagnosis was therefore based on glucose values from HemoCue. HemoCue may, however, give falsely low glucose levels relative to the triglyceride content ¹⁷⁴. A study concluded that if triglycerides were > 3.0 mmol/l, they might interfere with HemoCue measured glucose ¹⁷⁴. However, in our data, triglycerides seemed to interfere with glucose measured by HemoCue in a linear manner (Table 9). As pregnant women have higher triglyceride levels than non-pregnant women, this interaction is even more topical in our data. The choice of measurement method was therefore crucial in relation to the interpretation of findings.

Table 9. Difference in glucose levels measured with HemoCue and Vitros across quartiles of triglycerides.

	Quartiles of fasting triglyceride levels (mmol/l)				P ¹
	1 (0.52-1.48)	2 (1.49-1.87)	3 (1.88-2.34)	4 (2.34-5.60)	
HemoCue-Vitros (mmol/l)					
ΔFasting glucose	0.53±0.45	0.49±0.41	0.41±0.35	0.23±0.46	<0.001
Δ2-hour glucose	0.70±0.66	0.60±0.64	0.34±0.51	0.20±0.55	<0.001

Δ Fasting glucose is the difference in glucose levels measured with the two methods (HemoCue minus Vitros)

Δ 2-hour glucose is the difference in glucose levels measured with the two methods (HemoCue minus Vitros)

¹ ANOVA

The GDM variable, used as outcome in Paper 1 and 2, was based on glucose data analyzed with HemoCue. High triglyceride levels may have resulted in falsely low glucose levels, and thereby potentially a falsely low prevalence of GDM. However, the objectives in paper 1 and 2 were to explore whether weight gain or early pregnancy adiposity were risk factors for GDM, and not to estimate the true GDM prevalence. Interference between triglycerides and glucose levels in the analyses may, however, have weakened the strength of the associations in paper 1 and 2, as triglycerides levels were slightly higher in overweight and obese than in underweight or normal weight women.

In paper 3, we wanted to explore how maternal glucose and lipid levels influenced the birth weight and subcutaneous fat of the newborn offspring. As triglycerides were included in the regression model, we believe that using HemoCue values in this paper would bias the results, while Vitros values would give a more correct picture of the relationship between maternal glucose and offspring's birth weight and subcutaneous fat. Falsely low glucose values measured with the Vitros method will not change any of the relationships, although it is important to be aware that the observed glucose values may be too low.

The WHO 2013 GDM criteria demands the use of one or more abnormal glucose values (fasting, 1-hour or 2-hour glucose). If we had the 1-h glucose value available, the prevalence of GDM would probably be slightly higher. This may further contribute to a falsely low GDM prevalence, which also may weaken the strength of our associations.

Neonatal characteristics

Although the inter-rater variability for skinfold measurement was lower in the neonates than in the mothers, it was still relatively high. Again, we did not find any indications of bias, but the random errors may give imprecise measurements which, in turn, may weaken potential associations with the risk factor variables tested.

5.1.4 Confounding

Although we have adjusted for a selection of possible confounders that were measured in our cohort, and have performed sensitivity analyses to limit confounding, there will always be potential confounding in observational studies. BMI, GDM, weight gain, weight retention and growth of the foetus will all be influenced by maternal energy balance and thus maternal

physical activity and diet. Another important possible confounder to the ethnic differences in GDM is socio-economic position, which has been related to ethnic health differences in several studies. Socio-economic position is also highly related to lifestyle factors such as diet and physical activity. However, the objective of the three papers were not to explore whether diet, physical activity or socio-economic position explained the ethnic differences in adiposity, weight gain etc., but to explore whether ethnic differences in adiposity were able to explain some of the ethnic difference in risk of GDM, and whether the effects of maternal glucose, lipids and weight gain on offspring's birth weight and subcutaneous fat differed across ethnic groups.

5.1.5 Study design

The STORK Groruddalen study is a population-based prospective cohort study. Prospective cohort studies limit the risk of recall bias and reverse causation, but are at high risk for confounding. Still, a prospective cohort study was considered an appropriate study design to study risk factors associated with GDM across ethnic groups, as GDM is a quite prevalent outcome. The population-based design strengthens the external validity of the findings.

5.1.6 External validity

The sample should be representative for the main ethnic groups of pregnant women living in Groruddalen. Since the internal validity has been accounted for and the results have been interpreted with respect to potential bias, the findings of this thesis should be generalizable to pregnant women of the described ethnic groups, living in Norway. Since the effects of maternal adiposity and mid-gestational weight gain were independent of ethnicity, their relationship with GDM and neonatal anthropometry will probably be generalizable to other populations of healthy pregnant women.

5.2 DISCUSSION OF MAIN FINDINGS

5.2.1 Paper I – mid-gestational weight and fat gain and risk of GDM

We did not find any ethnic differences in weight gain. However, as the IOM recommendations for weight gain do not take ethnicity into account although they use pre-pregnancy BMI to classify maternal adiposity, the IOM recommendations may not be adequate for all ethnic groups, especially Asians. Due to a higher degree of adiposity for the same BMI in Asians, the recommended weight gain may be too high for Asians.

Our results suggested that central fat gain from 15 to 28 weeks' gestation was a stronger risk factor for GDM than weight gain and total fat gain. Thereby, central adiposity may be of importance also in relation to GDM. This finding fits well with the hypothesis that ectopic fat accumulation may play a central role in the development of diabetes. Our findings also fit well with the idea of a relationship between central adiposity and hepatic insulin resistance as the WHO 2013 GDM criteria especially capture those with elevated fasting glucose, characteristically associated with hepatic insulin resistance.

In paper 1, we showed that the effects of weight, total and central fat gain on GDM remained after adjusting for HOMA-IR in early pregnancy. However, insulin resistance explained some of the ethnic differences in GDM risk, especially among South Asians, indicating that at least some of the ethnic differences in GDM risk may be due to differences in insulin resistance in early pregnancy. Insulin resistance in early pregnancy did, however, not entirely explain the higher GDM risk in South Asians. Since HOMA-IR is not the gold standard and merely an indicator of insulin resistance, we cannot rule out that the remaining higher risk for GDM among South Asians is due to differences in insulin resistance. In fact, studies using gold standard methods for assessing central adiposity and insulin resistance found that central adiposity, as well as general adiposity, contributed greatly to the metabolic disturbances observed in South Asian men, especially to the increased insulin resistance^{175 176}. Hence, finding the reason to the increased insulin resistance in the non-Western ethnic groups may be important in finding explanations for the ethnic differences in GDM and T2DM risk. One study found higher levels of liver fat in South Asians than “Caucasians”¹⁷⁷, which may explain their observed higher insulin resistance.

One study showed that short term intake of a high fat high calorie diet induced insulin resistance in South Asians, but not in “Caucasians”¹⁷⁸, indicating that there might be ethnic differences in how well different diets are tolerated. As many South Asians tend to adopt a Westernized version of their traditional diet¹⁷⁹, this could be one reason to why South Asians have a higher risk of GDM and T2DM.

5.2.2 Paper II – ethnic differences in subcutaneous fat and s-leptin and GDM

In paper 2 we found higher amounts of subcutaneous fat, as measured with skinfolds, in South Asians than in Europeans, despite a lower BMI. Two of these skinfolds, subscapular and suprailiac, are more closely associated with central adiposity⁶⁵ and thereby suggest that the South Asian women in our study had more central adiposity than Europeans. However, studies using the gold standard methods for measuring central adiposity differ in their findings. One study found no difference in superficial or deep subcutaneous fat or central adiposity between Pakistanis and Norwegians¹⁸⁰, although statistical type 2 errors cannot be ruled out due to small numbers. One study found higher levels of deep subcutaneous fat in South Asians than in Europeans¹⁸¹ while another study found lower levels of superficial subcutaneous fat in South Asians than in “Caucasians”¹⁷⁷.

We found higher s-leptin levels for a given BMI in South Asians, supported by several others^{53,54,182,183}. The higher s-leptin observed in South Asians could be a further indication of poor measurement techniques for adiposity, as s-leptin is known to reflect fat mass. However, there are other possible explanations to the higher s-leptin observed in South Asians.

A higher secretion of leptin could be caused by hypertrophic adipocytes¹⁸⁴. South Asians have been found to have larger adipocytes than “Caucasians”^{177,185}, and hypertrophic adipocytes have, in turn, been associated with insulin resistance^{184,186,187} and T2DM¹⁸⁶. The kidneys account for approximately 80 % of leptin removal from plasma¹⁸⁸. However, leptin clearance do not seem to increase in obese individuals, resulting in a lower fractional removal rate with increasing leptin levels¹⁸⁸, which partly may explain the high s-leptin levels observed in obese individuals. South Asians with T2DM have been found to have a higher incidence and faster progression of renal disease¹⁸⁹. Although highly speculative and not yet

studied, South Asians may have a lower renal function which in turn may lead to reduced leptin clearance, which could explain their higher s-leptin.

Banks and co-workers convincingly found that high triglyceride levels, either induced by starvation or diet-induced obesity, decreased transport of leptin across the blood-brain barrier, and treatment with gemfibrozil reversed both the hypertriglyceridaemia and the reduced leptin transport across the blood-brain barrier in mice ¹⁹⁰. If leptin is not transported across the blood-brain barrier it may not induce satiety, which may explain the paradox of high s-leptin levels in obese as they often have elevated triglyceride levels. As higher triglyceride levels have been observed in South Asians compared to Europeans ¹³⁰⁻¹³², it is possible that South Asians have reduced leptin transport across the blood-brain barrier and thereby reduced satiety. Also, the concentration of soluble leptin receptor seems to be important in bioavailability of leptin, as low levels of the soluble leptin receptor are associated with obesity ^{43,191,192}. Weight loss, on the other hand is associated with an increase in the soluble leptin receptor concentration ^{43,193}. A strong inverse association has been found between plasma soluble leptin receptor levels and T2DM ¹⁹⁴. Although scarcely explored, a study found ethnic differences in the concentration of soluble leptin receptor, with lower levels observed in Japanese and Chinese compared with “Caucasian” women ¹⁹⁵. Soluble leptin receptor levels in South Asians have, to my knowledge, not been studied.

Hence, the high s-leptin observed in South Asians may be a result of several mechanisms, such as hypertrophic adipocytes, lower leptin clearance, differences in soluble leptin receptor or simply due to high amounts of fat relative to low amounts of lean mass and body size.

5.2.3 Paper III – effects on offspring’s birth weight and subcutaneous fat

Mid-gestational weight gain was a stronger predictor of both birth weight and subcutaneous fat in the newborn than maternal glucose or lipids. The excessive weight gain is an indication of maternal energy excess, and the energy excess is most likely transferred to the foetus to some extent. There are several ways in which the foetus receives energy from the mother, such as through amino acids, NEFAs, ketone bodies or other substrates in addition to the substrates measured in our study. However, although our study cannot designate the exact mechanisms in which weight gain affects neonatal anthropometry, our study shows that mid-

gestational weight gain may be a better predictor of birth weight and neonatal subcutaneous fat than maternal glucose, lipid levels, or pre-pregnancy BMI.

We used birth weight and neonatal sum of skinfolds as proxy outcomes for future health. Although there is some evidence suggesting that high birth weight increases risk for adult overweight and T2DM ^{152,153}, the long-term effects of high amounts of subcutaneous fat in the newborn seem to be poorly studied. However, offspring of diabetic mothers—which are generally born with more adipose tissue than offspring of non-diabetic mothers—have increased risk of obesity and diabetes in adulthood ¹⁵. Also excessive maternal weight gain has been associated with poor adult health in the offspring ⁷². Whether this is due to increased neonatal adiposity, however, is not fully explored.

In accordance with the developmental overnutrition hypothesis ⁹¹, Dabelea and Crume ¹⁹⁶ express their concern that maternal pre-existing weight and weight gain during pregnancy may “generate an intergenerational vicious cycle of obesity and diabetes because heavier mothers give birth to heavier daughters, who are at increased risk to be obese themselves during their reproductive years, thus perpetuating the cycle” ¹⁹⁶. The vicious cycle of obesity and diabetes may therefore be enforced by pre-existing overweight and obesity, excessive weight gain during pregnancy and GDM, which may increase the risk of overweight, obesity and diabetes in the adult offspring.

Epigenetic changes in the foetus in response to maternal exposure have been proposed as potential mechanisms for intrauterine programming. Very little research in this field has been performed in humans, and most studies that supports an epigenetic role in programming of the foetus has been as a result of undernutrition.

5.2.4 Public health implications

The high level of adiposity, the high mid-gestational weight gain and their relationship with risk of GDM, birth weight and neonatal subcutaneous fat, suggest a great potential for prevention. In light of the developmental overnutrition hypothesis, ethnic differences in GDM, and weight and fat retention after delivery may contribute to ethnic differences in poor health, as the exposed offspring may be at higher risk of obesity and diabetes than the un-exposed. We did not find ethnic differences in mid-gestational weight gain, but prevention of excessive weight gain may still reduce the risk of GDM and the birth weight and

subcutaneous fat of the offspring. The higher amounts of subcutaneous fat and s-leptin in South Asians explained some of the higher GDM risk in South Asians than in Europeans.

Randomized trials have shown that treatment of GDM reduces neonatal fat mass, birth weight and macrosomia^{197,198}, perinatal morbidity¹⁹⁷, and also improve maternal outcomes^{197,198}. A recent study found no reduction in childhood obesity or metabolic dysfunction when studying long-term effects in offspring born to mothers who developed GDM¹⁹⁹, although they found an effect on neonatal measurements. However, as the authors mention, the study was probably statistically under powered¹⁹⁹.

Although the IADPSG criteria has been criticized for labelling a large proportion of pregnant as ill, a recent study concluded that application of the IADPSG criteria significantly reduced adverse pregnancy outcomes and proved cost-effective compared to the conventional Carpenter-Coustan criteria, despite a 3.5 fold increase in GDM prevalence²⁰⁰. This finding indicates that detection of less severe hyperglycaemia and offering lifestyle advice to manage the hyperglycaemia may improve health for both the mother and her offspring. Since GDM is such a prevalent outcome, detection and management of GDM may thus have a large impact on the public health.

Studies exploring the effect of lifestyle interventions on weight gain and maternal and foetal outcomes have given different results. Thangiratinam²⁰¹ found in a meta-analysis of randomized controlled trials that among lifestyle interventions on maternal weight and obstetric outcomes, dietary interventions seem to be the most successful²⁰¹. Physical activity interventions, on the other hand, seem to be less effective²⁰². Dietary intervention during pregnancy significantly reduced weight gain in pregnancy, GDM, pre-eclampsia, gestational hypertension and preterm delivery for the mother²⁰¹. For the offspring, dietary intervention during pregnancy reduced risk of shoulder dystocia and border significantly reduced risk of intrauterine death²⁰¹. Two recent randomized controlled trials confirmed that lifestyle intervention during pregnancy may reduce gestational weight gain^{203,204}. Other randomized controlled trials have showed that low intensity intervention successfully limited postpartum weight retention^{205,206}. Although there are some indications pointing towards a benefit from lifestyle interventions during pregnancy, the clinical trials to explore the effect of lifestyle interventions on weight gain and other obstetric outcomes are few, heterogeneous and criticized of being of poor quality²⁰⁷. Intervention trials exploring the effect of weight loss in overweight and obese women prior to conception on obstetric outcomes are lacking.

5.2.5 Ethical hesitations to recommending weight loss

The findings in this thesis suggest that promoting a healthy pre-pregnancy body weight and a healthy weight gain during pregnancy, may reduce risk of GDM, high birth weight and neonatal adiposity. However, for the past years, there has been a debate concerning whether overweight and obesity per se is detrimental to health, as a systematic review and meta-analysis suggested that overweight may be protective in relation to morbidity and mortality²⁰⁸. Further, a study concluded that being labelled as overweight or obese during adolescence was a significant predictor of future risk of obesity, independently of BMI at time of weight labelling²⁰⁹, suggesting that stigmatization may be more detrimental than the excessive weight per se.

However, several studies have busted the myth that metabolically healthy obesity exists²¹⁰⁻²¹⁴. Although metabolically healthy obese individuals have lower risk of morbidity and mortality than their metabolically unhealthy counterparts, the metabolically healthy obese individuals have increased risk of diabetes²¹⁰⁻²¹³ and cardiovascular disease²¹² compared to metabolically healthy normal weight individuals.

As weight loss, and especially long-term maintenance of weight loss, is difficult, there are concerns to whether a focus on weight loss will improve health, especially since some studies suggest that weight fluctuation, results in higher morbidity and mortality²¹⁵⁻²¹⁷. However, a study found that these associations between weight fluctuation, morbidity and mortality were attributed to disadvantageous lifestyle and pre-existing disease rather than the weight fluctuation itself²¹⁸. Either way, avoiding the initial development of obesity seems at present the best way to avoid the associated morbidity and mortality.

6 CONCLUSIONS

- Women of European, South Asian, Middle Eastern, African and East Asian origin experienced similar mid-gestational weight gain
- Mid-gestational weight gain, especially gain of central fat, was positively associated with increased risk of GDM
- Early pregnancy BMI, subcutaneous fat and leptin were all positively associated with increased risk of GDM
- Women of South Asian origin had more subcutaneous fat and higher s-leptin for the same BMI as Europeans
- Women of South Asian origin retained more weight and fat after delivery than Europeans, potentially leaving them at even higher risk of GDM in future pregnancies
- Maternal glucose and lipid levels were positively associated with offspring's birth weight and subcutaneous fat
- Mid-gestational weight gain explained some, but not all, of the effect of glucose and lipids on offspring's birth weight and subcutaneous fat
- Mid-gestational weight gain was a stronger predictor of both birth weight and

Maternal adiposity did not fully explain why some ethnic groups, especially South Asians, have a higher risk of GDM, but ethnic differences in insulin resistance in early pregnancy may play an important role.

Prevention of overweight and obesity in women of fertile age would probably be of major public benefit with regards to the future health of the mothers and their babies. The stronger effect of mid-gestational weight gain than maternal glucose or lipids on offspring anthropometry, suggest that preventing excessive mid-gestational weight gain may be more important than preventing GDM. However, they generally depend on the same preventive strategies, with a healthy diet without energy excess and increased physical activity.

Pregnancy is often considered a window of opportunity to attain a healthy lifestyle, as pregnant women generally are highly susceptible to lifestyle advice. Health workers probably should have a larger focus on a healthy weight gain during pregnancy to reduce the risk of GDM and its consequences, as well as emphasize the importance of avoiding weight retention after pregnancy to secure a good starting point for future pregnancies.

7 FUTURE RESEARCH

Future studies should explore whether total or regional adiposity can explain ethnic differences in insulin resistance while using more accurate measurements of fat than in the present studies. Especially we suggest to explore whether total and regional adiposity, as well as liver fat and pancreatic fat, can explain the insulin resistance, as measured by hyperinsulinaemic euglycaemic clamp, across ethnic groups.

Soluble leptin receptors seem to be protective of T2DM, and are inversely associated with adiposity. Future research should explore whether ethnic differences in soluble leptin receptor are present, and whether ethnic differences in soluble leptin receptor can predict the higher GDM risk in South Asians. Levels of soluble leptin receptors or the ratio between soluble leptin receptors and s-leptin, may be more precise predictors of GDM or T2DM risk than s-leptin isolated, especially across ethnicity.

It would be interesting to explore whether epigenetic modifications of maternal and/or foetal tissues may explain the transfer of maternal adiposity and weight gain to the foetus

Few studies have explored the long term health consequences of being born with large amounts of subcutaneous fat, and this may be addressed through long-term follow-up of well-characterized birth cohorts.

Finally, intervention studies in multi-ethnic cohorts of pre-pregnant women or women in early pregnancy to improve physical fitness and reduce overweight and obesity would be of high value.

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